P-2000

Laser Based Micropipette Puller
Optical Fiber Puller

OPERATING MANUAL (Before upgrade)

(Rev. 3/92)

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P-2000 Quartz Puller
Operating Manual

UNPACKING AND SETTING UP

The Model P-2000 is shipped to you in a prefabricated foam mold. Please take note of this method of packaging. Should it ever be necessary to ship the puller to another location, the same method of packaging should be employed.(Additional packing material may be purchased from Sutter Instruments.)

IMPORTANT: Improper packaging is a form of abuse, and as such can be responsible for voiding the warranty where shipping damage is sustained as a result of such packing.

Place the puller in a location where there is a free flow of fresh air on all sides. The fan draws air in through the vent on the back of the instrument and exhausts it out from the top plate. NEVER ALLOW THE FREE FLOW OF AIR TO BE RESTRICTED.

Since the P-2000 is a microprocessor controlled device, it should be accorded the same system wiring precautions as any 'computer type' system. If microprocessor based systems in the lab require line surge protection for proper operation, then the same protection should be provided for the P-2000.

FIRST TIME USE

Plug the instrument into a power receptacle of the correct voltage and frequency. NOTE: A power source that is essentially electrically noise free is desirable. The control circuitry of the puller uses digital logic that may be susceptible to transient spikes that can be caused by faulty wiring or noise producing machinery, such as centrifuges or other equipment utilizing SCR control circuitry on the same power lines.

In order to protect the tilting mechanism associated with the scanning mirror, a shipping screw has been installed next to the micrometer located at the back of the instrument. This screw is turned down prior to shipping to unload the micrometer and lock the table in place. Before using the puller, run this screw up (turn counter-clockwise) until it is nearly out of the baseplate. There should be a factory default setting indicated near the micrometer. Make sure that the micrometer is set as indicated. If you are not sure how to read a micrometer, consult the maintenance section.

Programs 0 and 1 are preprogrammed into the puller by the manufacturer. Program 0 was developed using 1mm O.D. fused silica tubing with an I.D. of 0.7mm. Program 0 will pull a

micropipette of less than .05 micron. Program 1 is for pulling optical fibers and was developed using 125 micron single mode fibers with a 10 micron silica core. To change from one program to another press the reset button on the front panel, and then select the program on the keypad followed by the ENTR key. DO NOT USE OTHER PROGRAMS OR CHANGE THE VALUES IN THESE PROGRAMS UNTIL YOU HAVE READ SECTIONS II, III AND IV OF THIS MANUAL.

To execute a program, place a piece of glass tube or fiber in the puller bars (glass loading is described in the operation section of this manual) and press the PULL key on the keypad. If the glass does not separate within 15 seconds, press the STOP key to turn the laser off. Read the manual before proceeding with program or optical adjustments.

Should you plan on using the P-2000 with a glass fiber or tube that has been coated with any combustible material, it is highly recommended that you remove the coating from the glass prior to executing a pull. The smoke that is generated from the various combustible coatings is likely to coat the retroreflective mirror and interfere with proper performance of the P-2000.

SECTION II

MECHANICAL DESCRIPTION, CONTROLS AND ADJUSTMENTS

This section presents a basic mechanical description of the P-2000, with particular emphasis on terminology. Knowing the names of the various parts greatly facilitates communication between the investigators and the manufacturer when discussing adjustments or service problems. In addition, various controls and adjustments on the top of the instrument are located and described. Those adjustments which are considered part of maintenance procedures are dealt with in Section V.

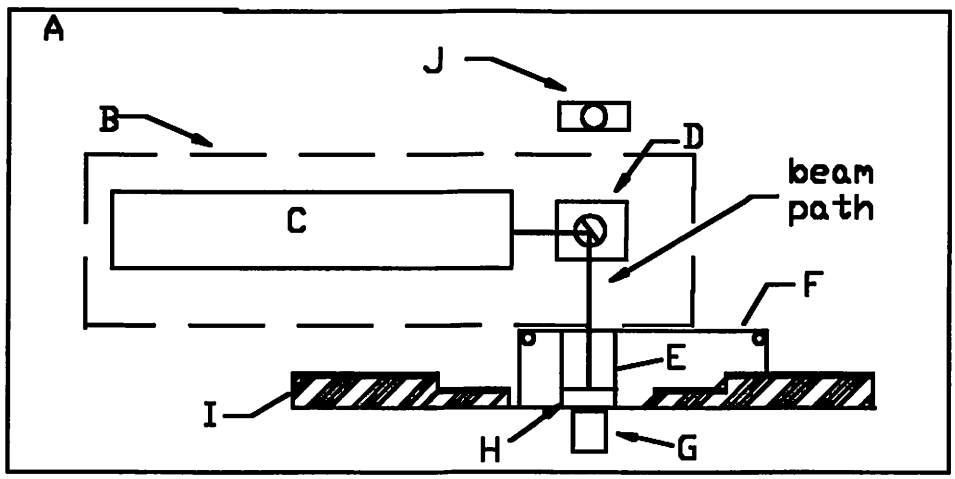


FIGURE 1

Top Plate: (Fig. 1a) The top is the metal plate on which is mounted the laser, puller panels, cover plate, etc.

Base: The base contains the laser power supply, transformers, circuit board and pull solenoid assembly.

Laser Enclosure: (Fig. 1b) This cover safely encloses the laser, the stepper motor and scanning mirror assembly.

Cover Plate: (Fig. 1f) The cover plate conceals the entry of the pulling cables into the base of the instrument. It is attached to the top by two screws, in slots, at points E and E'.

Panels, Left And Right: (Fig. 2k) The panels are the angled surfaces upon which are mounted the puller bars and their bearings, the spring stops, the bumpers, and the upper cable pulley assemblies. Except for minor differences in shape, the left and right panels are identical. Note the three socket-head cap screws that attach each panel to the top.

These screws are used to align the pullers bars. Puller bar alignment is covered in the maintenance section.

Puller Bars: (Fig. 2r) This assembly consists of the puller bar, glass clamp, clamping knob and cable retaining screw. The puller bar is made of mild steel and coated with a controlled thickness of hard chrome. Glass is loaded into the V groove near the tip of the puller bar and is held in position by tightening down the clamp knob. Pullers modified for pulling optical fibers have a shallow slit in the bars rather than a groove. The cable retaining screw holds the cable in a shallow groove at the end of the puller bar, and forms the 'resistance' against which the cable ends pull.

V-bearings: (Fig. 2 s) These bearings are the guides for puller bar motion. They are made of stainless steel and should NEVER be oiled (see maintenance section). Note that these bearings are mounted on stainless steel bushings one of which is round, and the other two being hexagonal. The hexagonal bushings are used to adjust position and ease of travel of the puller bars (see maintenance section).

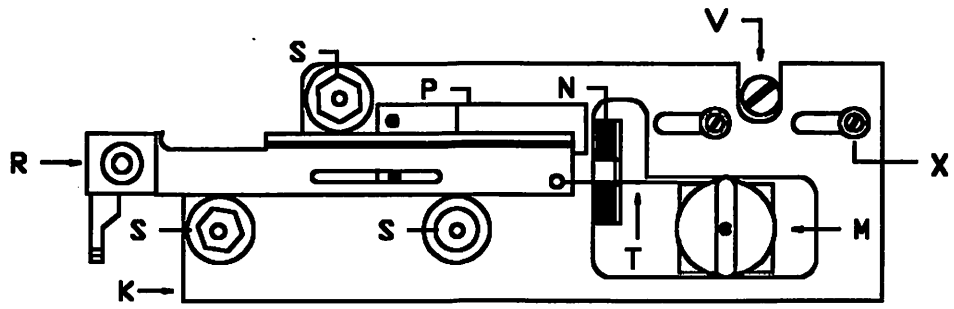


FIGURE 2

Bumpers: (Fig. 2 n) The bumper stops the motion of its associated puller bar and prevents impact forces from breaking the tips.

Spring Stops:(Fig.2 p) The spring stops are one way catches that prevent pipette tip collision by catching the puller bars as they rebound off the bumbers.

Upper Cable Pulley Assembly: (Fig. 2 m) This assembly conducts the pulling cables from the puller bars to the centrally located (and concealed) lower pulley assembly. Note that this assembly is attached to its panel by two screws, in slots, and contains a large eccentric adjustment screw. This eccentric screw is used to adjust cable 'tension'. Its use is covered in the maintenance section.

Pull Cable: (Fig.2 t) This cable conducts the pulling force of the solenoid to the puller bars via the upper and lower pulley assemblies. It is made of flexible metal with a nylon coating. NEVER PINCH OR DISTORT THE CABLE. The cable is terminated with crimped-on clamps or 'swages'.

Mirror Enclosure: (Fig. 1 e) This enclosure houses the retroreflective mirror and prevents user access to the laser beam. All glass heating occurs within this enclosure. Fiber pullers have a diagonal slit in the enclosure which facilitates loading fibers into the puller bars. The enclosure is attached to the cover plate with two slot head screws. The enclosure can be removed to allow access to the retro-reflective mirror (see maintenance section). An interlock switch prevents the laser from operating in the event the enclosure is removed.

Manipulator (Fig.1 g): The two stage manipulator is used to adjust the position of the retrorelective mirror.

Optical components:

LASER (Fig. 1 c): A 10 watt CO_2 laser provides the heat source for melting the glass. Laser output power is regulated by feedback control electronics. The laser output power is specified by the program value entered for HEAT.

Scanning Mirror(Fig. 1 d): The laser beam is projected onto the back face of the glass by a reflective mirror. This mirror is mounted on a rotating base driven by a stepper motor. Heat application to the glass is achieved by repeatedly scanning the mirror between the limits of a defined longitudinal area.

Scanning Mirror Tilt Micrometer (Fig. 1 j): The scanning mirror is mounted on a tilting table. The tilt controls the elevation of the laser beam relative to the glass. The degree of tilt is controlled by the micrometer located at the back of the puller. In order to protect the tilting mechanism, a shipping screw has been installed next to the micrometer. This screw is turned down prior to shipping to unload the micrometer and lock the table in place. Before using the puller, run this screw up (turn counter-clockwise) until it is nearly out of the baseplate. There should be a factory default setting indicated near the micrometer. Make sure that the micrometer is set as indicated. If you are not sure how to read a micrometer, consult the text in the maintenance section.

Retroreflective Mirror(Fig. 1 h): The portion of the beam that is projected past the tubing is collected by a reflective concave mirror mounted behing the tubing. This retroreflective mirror redirects the divergent laser radiation onto the back side of the glass and thereby

provides relatively uniform heating around the circumference of the glass.

Optical Alignment:

Proper alignment of both the front scanning mirror and rear collector mirror is critical for optimizing the performance of the laser puller. Both mirrors have been adjusted for optimum performance using 1mm outside diameter glass (or 125 micron fibers for special fiber pullers). If you will be using larger diameter glass, please read the comments on glass size in Section IV. We recommend that you refrain from adjusting the mirrors until you have spoken with a member of the Sutter Instrument technical staff. In the event that you do need to adjust the mirrors, consult the maintenance section for instructions

SECTION III

FRONT PANEL DESCRIPTION AND OPERATION

The aim of this section is to provide the user with the information necessary to operate the P-2000. It begins with some important definitions and descriptions of the front panel controls.

DEFINITIONS

Program: A program consists of one or more cycles, which when executed in sequence will 'pull' the capillary glass inserted in the instrument. A program can be up to 16 cycles in length.

Cycle: A cycle consists of a HEAT value, a FIL(FILAMENT) number, a VEL.(VELOCITY) value, a DELAY value and a PULL value.

Loop: A loop consists of one or more cycles that are repeated by the instrument until glass separation is achieved.

Ramp Test: A ROM resident program designed to facilitate program alterations when selecting different filaments and/or when using different glass tubing. The test is described in subsequent text.

FRONT PANEL CONTROLS

Reset: When momentarily pushed, this switch resets the microprocessor to an initialized condition. It is used to change from one program to another, and in other circumstances as described below.

Keypad: There are three 'groupings' of keys on the keypad: numerical/decision, editing and control. They function as follows:

Numerical/Decision (0-9): These keys are used to enter the number of the program being chosen, the various values for HEAT, PULL etc. and to make yes/no (1/0) decisions in certain situations.

Editing (CLR, ENT, NEXT, LAST): These keys are used for entering, deleting and editing programs. They allow one to move forward and back through a program, enter new values, and clear out unwanted values. In addition, the CLR key is the access key to the Ramp Test. The function of the various keys will be explained in the context of instrument operation in subsequent text.

Control (PULL, STOP): These keys control the initiation and cessation of program execution and control of the Ramp Test.

DISPLAY PARAMETERS:

HEAT (Range - ramp minimum to 999): Specifies the output power of the laser, and consequently the amount of energy supplied to the glass. Generally changes will be made in steps of about 10 units since in most cases smaller changes will have no effect.

FILAMENT (Range- 0 to 15): Heat is supplied to the glass by longitudinally scanning the laser beam onto the glass in a length and rate dependent manner. The P-2000 is preprogrammed with 16 different scanning patterns, each of which constitues a different filament. Filaments vary both in the length of glass heated and the scan rate within this length. The length of the area scanned is analagous to the width of a conventional metal heating filament. The scan rate relates to the laser power (heat) distribution, but at this point in time, the relationship between the scan rate and the glass pulling process is not well understood. The following charts lists the relationship between a selected filament number and the length and rate of the beam scan.

Filament #	0	1,6,11	2,7,12	3,8,13	4,9,14	5,10,15
Scan Length	1mm	1.5	1.9	4.5	6.5	8

Filament	0	1-5	6-10	11-15
Scan rate	no scan	high	medium	slow

VELOCITY (Range- 1 to 255): The velocity of the system is measured as the glass softens and begins to pull apart under a constant load. The increasing velocity of the initial pull is determined by the viscosity of the glass, which in turn is a function of the glass temperature. The adjustable velocity allows for selection of a precise glass temperature as the trip point for the hard pull. One unit represents a change of one or more millivolts of transducer output depending on the transducer being used. Useful values for velocity range from 10 to 100 with the lower values being used for fibers and higher values for micropipettes.

DELAY(Range- 1 to 255): The DEL variable controls the timing of the start of the hard pull relative to the deactivation of the laser. An increase in the value of DEL will cause the glass to be cooler when the hard pull occurs. Thus shorter pipettes may be obtained by increasing DEL.

The actual significance of the DEL is slightly more involved than a simple delay. For a DEL value of 128, the hard pull will turn on just as the heat turns off. Each additional unit of DEL will produce a 1 msec delay of the hard pull. Thus at the maximum value of 255, the hard pull will be delayed by 127 msec. Values below 128 advance the onset of the hard pull relative to the end of heat so that the hard pull will start prior to the end of heating. For each unit that DEL is decreased below 128, the heat will remain on for about 1 msec after the start of the hard pull. For the minimum value of DEL 1, the heat will therefore remain on for 127 msec after the hard pull has turned on.

PULL (Range-0 to 255): A change of one unit represents a change of 4 milliamps of current through the pull solenoid. In the case of pull strength useful changes are 10 units or more to see an effect.

SECTION IV

OPERATION

Apply power to the instrument. After an automatic 'power on' reset, the display will appear as follows:

WHAT PROGRAM DO YOU WANT TO USE

The P-2000 is shipped with two programs already stored in memory. To select a program, enter the program number followed by the ENTR key. Program 1 is for a fused silica optical fiber. Load a length of fiber into position. This is best done by first sliding the fiber into the slit in the mirror enclosure. Loosen the glass clamp on the right or left puller bar, place the fiber in the slit in the puller bar and firmly tighten down the clamp. Release both puller bars (if they are latched by the spring stops) and pull them to the center until they stop. Now place the opposite side of the fiber into the slit on the opposite puller bar and tighten the glass clamp. **It is important that the fiber be positioned in the slit in both puller bars.** Press the 1 key followed by ENTR on the keypad; line 1 of Program 1 will appear. Press the PULL key and the puller will execute program 1. Remove the pulled fibers from the glass clamps and press the RESET button.

Once again the display shows the message:
WHAT PROGRAM DO YOU WANT TO USE

At this point there are three options: 1) Choose a program, load glass and execute the pull; 2) Create a new program or edit an old one; 3) Run the Ramp Test. The user should now create a program. (please note that the programs that will be written in the following text are not meant to pull pipettes, but are intended as an exercise to help develop an understanding of the programming process).

First press a key other than 0 or 1. The display should come up with no values for HEAT, PULL, etc. If there are already numbers or symbols entered as program values, make sure that this program was not entered by another user of the puller. Unused program areas are usually cleared before a puller is shipped, but occasionally random values or test program values are inadvertently left in memory. The clear function is outlined below.

CLEAR: When a new program is being entered into memory in an area occupied by another program it is helpful to be able to 'clean out' the old program. Also, it may be desirable to remove all the values from the last cycles of a long multi-cycle program to allow for fine tuning of these final

cycles. This clearing of program values is accomplished by the CLR key.

The CLR key sets all values to 0 from the line on which the cursor is located to the end of the program. Thus, if the cursor is on line 05 of a seven line program and your response to the query:

DO YOU WISH TO CLEAR ALL VALUES FROM THE PRESENT LINE TO THE END OF THE PROGRAM YES= 1, NO= 0

is a '1'. Only the values up through line 04 will remain intact. Clearing out a whole program simply requires that the cursor be on line 01 before the 'yes' response.

RAMP TEST

The 'no' or '0' response to the above question provides access to the Ramp Test and the following display:

DO YOU WANT TO PERFORM A TEST?
NO= 0, RAMP= 1

A '0' response returns the user to the current program. A '1' response enters the Ramp Test. The next display after entering '1' is:

LOAD GLASS, CLAMP AND PRESS PULL

Load a length of capillary glass and the press the PULL key. The following events take place: 1) The laser is turned on at full power for 250 msec. after which the command voltage is decreased until the power output from the laser reaches a minimum criteria; 2) The laser command voltage is set to correspond to a Heat value of 150; 3) The mirror is rotated to focus the beam on the glass, and laser command voltage is incremented. The glass will begin to soften, causing the puller bars move apart with increasing velocity; 4) When a criteria velocity is achieved, the laser is turned off and the laser heat value (ramp test value) and minimum heat value are reported.

Press RESET to exit from the Ramp Test display, and return to the program select display.

It is important to note that at this time, the Ramp test is still under development. The intent of the test is to establish the minimum value for Heat which results with instability in the laser output and to establish a ramp value. Ramp test operation requires that a considerable amount of power be dissipated by a field effect transitor gating the laser. **You are advised to avoid running consecutive Ramp tests which might tax the thermal resistance of the FET.**

PARAMETER ADJUSTMENTS

Micropipettes programs are sufficiently different from optical fiber programs that the following information on parameter adjustments has been divided into two sections. If your research only requires one type of pipette, we recommend that you read through both sections for full appreciation of the capabilities of the puller.

I. MICROPIPETTE FABRICATION

HEAT (Laser output power)

The heat setting will affect the length and tip size of the pipette. Generally speaking, higher heat settings tend to give longer and finer tips. The recommended starting heat value is the ramp test value. Values lower than 200 should not be used. Timing the pulling cycle can provide the user with very useful information for developing pulling programs. At a heat setting of ramp value plus 15, a 1mm O.D., 0.50mm I.D. quartz capillary tube will typically pull in 4 to 6 seconds after the PULL key is pressed. If the pull takes longer than eight seconds, and you are trying to pull a fine micropipette, increase the heat in 10 unit increments until the pull takes place in less than eight seconds. If the pull occurs in less than three seconds, decrease the heat until the pull takes place in 4-8 seconds. For the best micropipette reproducibility with the finest tips, you should select a heat value that melts the glass in 4 to 5 seconds. If the pull takes 10 - 15 seconds, there will be inherently more variablity in laser operation and pipette reproducibility will be compromised. In all cases, if the glass hasn't melted in less than 15 seconds, increase the heat.

FILAMENT

The filament selected will also affect the time it takes to pull an electrode. Any filament can be used to form a microelectrode, but wider filaments tend to work more effectively. If you find that a wide filament is not melting the glass, try dropping down to a narrower filament which results with more power applied per unit length of glass. If you use a multiple line program, you will probably discover that as the glass has drawn out, the direct application of heat to the glass becomes even more effective. However, if the beam is not well centered on the glass, as the glass draws out you may find that the program stalls because the glass lies outside of the effective region of the Gaussian beam profile. You can try using a narrower filament with a slow scan rate which will have the effect of applying more power to the glass.

PULL STRENGTH ADJUSTMENT

Low values of pull strength in the range of 40-75 will give larger tips, while settings between 150-250 give the smallest tips. The pull strength can be set to any value desired with no danger of damaging the instrument. Once the trip velocity is attained, the hard pull is turned on for 1 second after the programmed delay has elapsed.

VELOCITY ADJUSTMENT (trip point)

The velocity value determines the point at which the heat is turned off. Velocity reflects the speed at which the two carrier bars are moving. The lower the velocity value the slower the speed of the bars when the trip point occurs. At that time the heat is turned off, and after the programmed delay has occurred, the hard pull is turned on.
Although the functional range of velocity is from 1-255, the velocity value is typically set between 45 to 60 for quartz micropipettes. For borosilicate or aluminosilicate micropipettes, velocity values should be increased to 100 - 150.

In a multiple cycle program, it is possible for the glass to separate before the trip velocity is attained. Thus, the glass is subjected to direct heating as it is separating. Such an occurance can lead to difficulties in forming tips as well as lack of reproducibility. If you are using a one line looping program, try decreasing the velocity a few units at a time. If your program is a multiline program, decrease the velocity in the next to last line of the program. Decreasing the velocity will increase the amount of glass left in the last cycle of program, thus allowing the glass to attain the trip velocity before separating.

DELAY

The Delay controls the timing of the start of the hard pull relative to the end of heating by the laser. The longer the delay, the cooler the glass will be when the hard pull occurs. In most cases when using a glass other than quartz, DEL will be 200 or more for microelectrodes. For quartz pipettes, DEL will usually be between 100 and 140.

II. OPTICAL FIBER MICROTIP FABRICATION

Optical fiber programs differ significantly from micropipette programs. The following parameter values are recommended for pulling fibers with sub-micron tips.

HEAT

If the optical alignment is adjusted properly, the heat required to melt a fused silica optical fiber is typically in the 350-500 range. Higher heats (up to 800) are useful for forming fine tips. If the fiber consistently breaks off rather than pulling apart, the heat should be increased.

FILAMENT

Concentrated heat seems to work best when pulling fibers. Thus, Filament 0 is recommended, which inhibits beam scanning. Wider filaments may be used to produce a more gradual taper, but avoid using delays of under 128 if you use filament other than 0.

VELOCITY

A velocity of 20 or less is recommended for pulling fibers. Values much above 20 will allow the glass to separate before the velocity is reached.

DELAY

Fibers cool very rapidly once the heat is turned off. If the fiber is too cool when the pull is executed, it will snap apart rather than drawing down into a tip. A delay of 128 causes the pull to start at the same time the heat is turned off. Decreasing the delay allows the heat to remain on as the pull starts. A delay of less than 124 seems to work best for optical fibers. The lower the delay, the longer the taper. Delays greater than 124 will produce shorter tapers. If the fibers break off rather than forming a tip, the delay is probably too high.

PULL

A Pull strength of 150 or greater is typically used for pulling fibers. Very high pull strength (>200) should produce the very finest tips, but they will be longer and less consistent as well.

USE OF LARGER DIAMETER GLASS

The optical alignment of the P-2000 is based on a 1mm diameter standard (or 125 micron fiber in the case of fiber

rubbing on the holes in the enclosure, it may still be possible to use without adjustment of the puller. The means of clamping the glass causes the center of larger diameter glasses to be displaced up and towards the user. This can place too much heat on the bottom of the glass, and can melt the glass away from the center of the laser beam resulting with a non-concentric pipette. An initial heating cycle should be used to draw the glass down to near 1 mm diameter. This will produce an hour-glass shape that is not well centered on the diameter of the original glass, but which may be worked in about the same way as 1 mm glass.

SECTION V

MAINTENANCE

A. Pulley Adjustment

The position of the two pulleys which carry the cables from the solenoid to the puller bars is adjustable. This adjustment should be made only if the two pipettes formed from one pull are of quite different lengths. This inequality may be caused by scanning mirror operating errors, inappropriate parameter values (too high a heat for a given velocity), but may also be caused by unequal cable tensions. The cable adjustment is made by moving one or both of the pulleys to equalize the tension on the two cables. It should be explained at this point that there are two sets of stops in the system. There are the stops (allen head screws) in the puller bars slots against which the bars rest, and a stop to prevent the solenoid from being pulled out of its housing. The adjustment of the pulleys must be made so that the puller bars will still come up against their stops while the solenoid is not against its stop. The two cables should not be under tension when the bars are against their stops, this is the position they would be in just before pulling a pipette.

You should be able to press on either cable between the puller bar and the pulley(Fig.2,t) and there should be about a 2mm deflection before the solenoid hits its stop. If the deflection is more or less, the pulley position should be changed. This is done by loosening the two screws above the pulley(Fig.2,x) and turning the chrome eccentric cam(Fig.2,v) to move the pulley in small increments until the two cables are of equal tension. If the puller bar no longer stops against its stop in the slot, but stops against the cable, then the cam must be adjusted back until the carrier once more hits its stop. It is important that the carriers come up against their stops with no tension on the cables. If there is tension, the initial pull will depend on how tightly you hold the finger stops when the glass is clamped in the puller bars. If this happens, the pipettes will not be consistent from pull to pull.

B. Cable Replacement

If the cable breaks or gets a bad kink, it will have to be replaced. This is done as follows:

Remove the two screws which hold the cover plate down (these are tamper-proof screws which require a special wrench for removal). The cover plate is the aluminum plate on top of

which the mirror enclosure assembly sits. With the two screws removed, the cover plate and mirror enclosure assembly can be lifted up and moved back out of the way. Below the cover plate the lower pulley assembly and the two cable wires are visible (Fig. 5 D, D').

To access the solenoid and the cable insertion points, you will need to lift the baseplate up off of the cabinet. Remove the 10 philips head screws from the perimeter of the baseplate. Lift the baseplate up off the cabinet a few inches and then tilt the front of the baseplate up as you would the hood of a car. The baseplate can be supported in this tilted position by resting the solenoid cage assembly (attached to the bottom of the baseplate) on the front edge of the cabinet

The next step is to remove the brass slug on the top of the solenoid (Fig.5,A). It is held on by two hex screws (Fig.5, B). The brass slug should now slide up and off of the shaft of the solenoid. The old cable can now be slipped out of the slot in the brass slug. At this point note the path of the cable as the new cable will be strung the same way as the old. To remove the old cable, use a wire cutter to cut the cable near the puller bars. Now pull out the cable. Slip the swaged end of the new cable in the brass slug and replace the brass slug. Be sure to get the hex screws tight. Both wires go through the first guide (Fig.5 C), and then each wire must be fed through a pulley(Fig.5, D,D') and its accompanying guides (Fig.5, E).
Using a small screwdriver, loosen the screw at the outboard end of the puller bar and remove the short piece of wire and its swage. Feed the wires through the outboard pulleys (Fig.5, F) so that the wires lie across the puller bars (Fig.5, G).

For the next steps, the puller bars must be held in toward the center. This can be done with a rubber band around the two finger bars to bring both bars in to the center. Loosen the two screws which lock down the two outboard pulleys and center the pulleys in their travel.
Now slip a swage on one of the wires. Pull on the wire to lift the solenoid. With the solenoid against its upper stop, position the swage over the hole (Fig.5, H)at the end of the puller bar and crimp the swage with a swaging tool. Using a wire cutter, cut the excess wire off and tighten the screw down on the wire. This must be done for each wire.

The baseplate can now be lowered down onto the cabinet and the screws reinserted. The cover plate and mirror enclosure assembly can also be reinstalled. The instructions for adjusting the outboard pulleys should now be followed to get the correct tension on the cables.

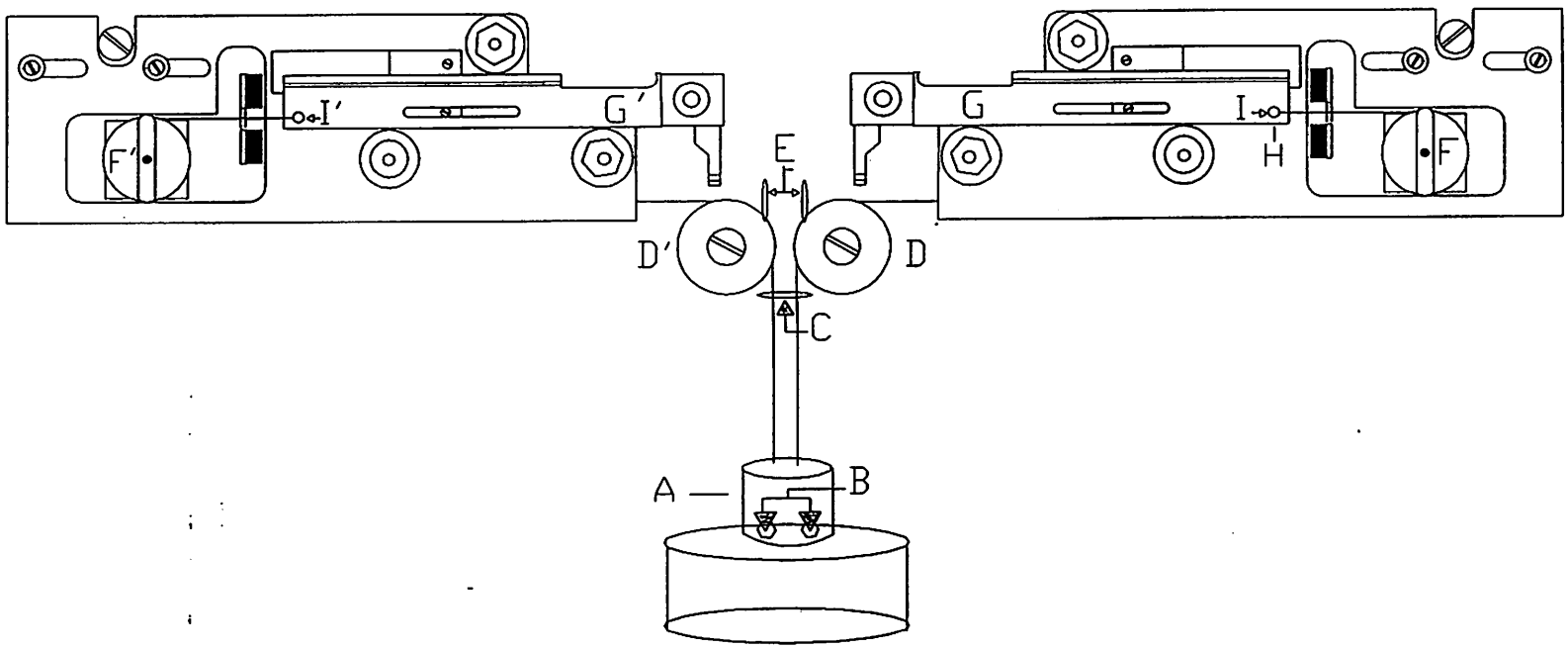


FIGURE 5

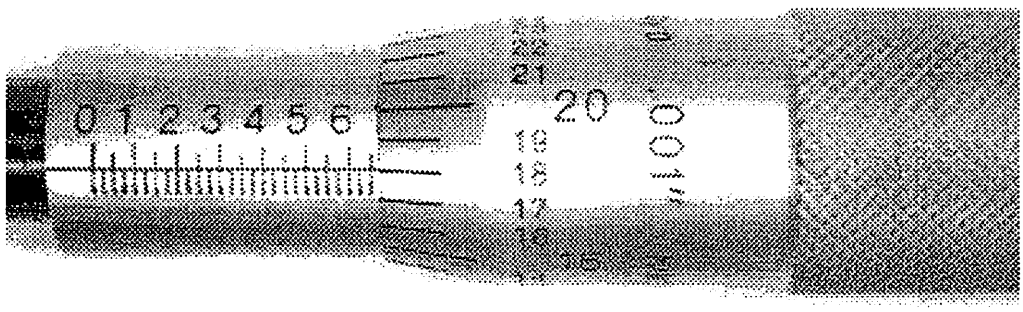
C: OPTICAL ALIGNMENT

Proper alignment of both the front scanning mirror and rear collector mirror is critical for optimizing the performance of the laser puller. Both mirrors were adjusted for optimum performance before shipping the P-2000 from the factory. **We recommend that you refrain from adjusting the mirrors until you have spoken with a member of the Sutter Instrument technical staff.** Optical alignment procedures for fibers are different than those for pipettes. Both procedures will be discussed.

SCANNING MIRROR TILT ADJUSTMENT

The scanning mirror is mounted on a tilting table. The tilt controls the elevation of the laser beam relative to the glass. The degree of tilt is controlled by a micrometer on the back of the puller. This micrometer pushes against an arm attached to the tilt table, which is spring-loaded against the micrometer. Turning the micrometer clockwise elevates the laser beam relative to the glass.

In order to protect the tilting mechanism, a shipping screw has been installed next to the micrometer. This screw is turned down prior to shipping to unload the micrometer and lock the table in place. Before using the puller, run this screw up (turn counter-clockwise) until it is nearly out of the baseplate. There should be a factory default setting indicated near the micrometer. Make sure that the micrometer is set as indicated. If you are not sure how to read a micrometer, consult the text below.



The micrometer is marked in 25 increments of 0.001" around its thimble and in 0.025" along its sleeve. The sleeve markings are numbered for each 0.1" and there is another mark to indicate 0.05". When the thimble is turned so that its zero mark is in line with the vertical line on the sleeve, the reading is an even increment of 0.025". Locate the highest 0.1" number visible and then add 0, 0.025, 0.05, or 0.075 to that number depending on the number of 0.025 increments visible between the 0.1" line and the edge of the

thimble. In this case the micrometer sleeve reveals 2 marks beyond 6 tenths of an inch, or .65" total.

If the thimble is rotated counter-clockwise from the 0 position, the number on the thimble next to the vertical line will increase, but no more 0.025" marks on the sleeve will appear until a full turn back to 0 has been made. The value from the thimble is added to the value on the sleeve to obtain the reading in .001". In this case the value is .018", which when added to .65" gives the final reading of .668".

The default setting supplied by Sutter Instrument should be adequate for most users. If you feel that this setting is not ideal, the micrometer allows for an easy change of tilt and a return to the old value. Since the micrometer is located about the same distance from the mirror as the target glass, a change in micrometer setting will produce about the same change in the elevation of the laser beam at the glass.

Because the scanning mirror and retroreflective mirror interact during the pulling process, you may find it difficult to isolate the effects of scanning mirror adjustments. If this is the case, you are advised to move the retroreflective mirror out, away from the glass before making further adjustments. As there is no sensor-based beam positioning system built into the P-2000, you will need to make beam position adjustments based on qualitative changes in the melted glass. By refering to rows 1 and 2 of the pipette profile chart that follows these instructions, you can very effectively adjust the beam position relative to the glass. Scanning mirror adjustments are made as follows:

1) Load a piece of quartz tubing into the puller.

2) Enter the following values into a program

HEAT- 700 to 800 FIL-1 VEL-250 DEL-200 PULL-0

3) Press PULL and after the glass has necked down or separated, note the geometry of the pipette tip. Refer to the chart at the end of this section for beam position adjustments as they relate to pipette tip geometry.

4) If the glass separated, load a new piece of tubing into the puller. If the glass only necked down, loosen the glass clamps and slide the glass along the puller bars until an unmelted section of glass is positioned in the beam path. Tighten the glass clamps.

5) Turn the tilt adjustment micrometer about 1/4 turn, then iteratively repeat the instructions from #3 on.

6) As viewed from the front of the puller, when the pipette geometry looks symmetric in the vertical plane, you should note the micrometer setting and proceed with the retro-mirror adjustment.

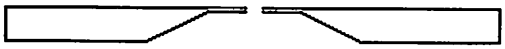
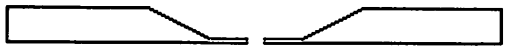
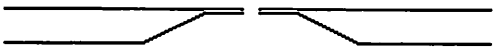
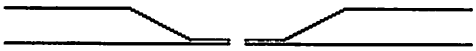
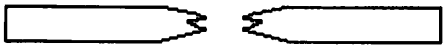
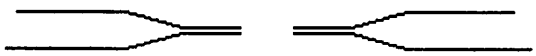
Retroreflective Mirror

The mirror is mounted on a two stage manipulator allowing you to adjust the up/down and in/out positions. When the scales on the left side of the manipulator are aligned at 0, the mirror will be in its factory calibrated position.

This manipulator should only be adjusted if the scales are not aligned or if the pipettes (or fibers) are noticeably asymmetric. To avoid accidental repositioning of the collector mirror, the manipulator has been fitted with 3/16 nuts which require a nut driver or wrench to rotate. Turning the top nut will change the up/down position of the mirror, while turning the nut facing you will change the in/out position of the mirror.

The manipulator travel limits are much greater than the travel limits of the mirror relative to the enclosure. Avoid exceeding the travel limits of the mirror as this may cause some damage to the manipulator.

The shape of the pulled pipettes is the best feedback for making adjustments to mirror position. To determine the laser beam position relative to a pulled pipette, it is useful to mark the pipette with an orientation mark before removing it from the glass clamp. The following chart lists some common pipette asymmetries and the corresponding mirror adjustments.

Pipette Geometry	Problem /Adjustment
 Pipette as viewed from **Front** of instrument	This condition results from too much heat being focused too **low**. Move mirror **up**.
Pipette as viewed from **Front** of instrument 	This condition results from too much heat being focused too **high**. Move mirror **down**.
A.  B.  Pipettes as viewed from **Top** of instrument	Either geometry indicates that the retro mirror is focused too close to the glass. Change the in/out position of the mirror. Try moving the mirror **in** towards the glass until you achieve a 'bird beak' geometry as illustrated in the next figure.
 Pipette as viewed from **Front** of instrument Same pipette viewed from the **Top**	This geometry indicates thatthe retro mirror is focusing the beam onto the glass. The 'bird beak' geometry is an indication that you are very close to optimum. Move the mirror **in** towards the glass to move the beam focus behind the glass.
 Pipette viewed from any side.	A nicely concentric pipette. The mirror is positioned properly.. Hide the nut wrench.

Pipette Geometry/Mirror Adjustment Chart

Optical alignment for Fibers

Because the laser beam is broad compared to the diameter of a fiber, the "tilt" setting is not critical However, since this must be set when the puller is unpacked, there may be some need for adjustment initially. In most cases, system realignment can be achieved by simply adjusting the tilt

micrometer. We recommend that you follow the instructions given previously for adjusting the scanning mirror using quartz tubing. If the tilt of the mirror is aligned for pipettes, it will be appropriately aligned for fibers.

The retroreflective mirror position is very sensitive, but the manipulator normally holds the factory setting through shipping without noticeable movement. Scales are provided on the manipulators. They are set to show 0 at the factory. Any fine adjustment will usually take less than one full turn of the nut on the manipulator.

Ascertaining the correct position of the retroreflective mirror is difficult for optical fibers. Please contact the technical support staff before making any adjustments. If retromirror adjustments are necessary, you may use the following procedure:

1)Load a length of fiber into the puller.

2) Enter the following values into a program

HEAT- 400 to 500 FIL-0 VEL-25 DEL-125 PULL-150

3) Press PULL. If the fiber rapidly melts and pulls apart, decrease the heat until you can establish a threshold heat level just below which the fiber will not separate. Load a fiber into the puller, press Pull, and as the fiber is heating, adjust the manipulator until you detect the highest concentration of heat at the fiber. This will be evident by the rate at which the fiber heats up and begins to draw apart. **Do not allow the laser to run for more than 30 seconds at a time.**

CLEANING THE RETROREFLECTIVE MIRROR

The retro mirror surface is close to the target glass and may become coated by materials liberated by the heating of the glass. This is very likely to happen if the glass is dirty, covered with oil from the fingers, or partially coated. The mirror is mounted in an enclosure that prevents operator access to the laser beam. We have fitted an interlock so that the laser is disabled if this enclosure is removed. Although an interlock has been provided, **the unit power cord must not be connected at any time while the enclosure is not in place! Damage to the unit may result if this caution is ignored! The enclosure is to be removed for maintainance only.**

The enclosure is fastened to a coverplate that is, in turn, fastened to the baseplate. Simply remove the 2 screws and the enclosure should lift out. **Make sure that you do not disturb the mirror or its adjustable mount.** The mirror is chrome plated, and may be cleaned with a soft, clean cloth

wetted with a solvent that produces no residue. You may be able to see a darkened area of contamination, which should come off quickly. Replace the enclosure and insert the screws. Before you tighten the screws check the position of the holes on the enclosure to insure that they will not interfere with the glass. Tighten the screws firmly before putting the unit back in service.

LIMITED WARRANTY

Sutter Instrument Company, a division of Sutter Instrument Corporation, limits the warranty on this instrument to repair or replacement of defective components for one year after the date of shipment, provided the instrument has been operated in accordance with the instructions outlined in the instruction manual.

Abuse, misuse or unauthorized repairs will void this warranty.

Limited warranty work will be performed only at the factory, the cost of shipment both ways to be borne by the user. This instrument is designed to pull glass pipettes for use on animal tissues. It is not intended to be used and should not be used in human experimentation or applied to humans in any way.

The limited warranty is as stated above and no implied or inferred liability for direct of consequential damages is intended.

DISCLAIMER
The pipette puller Model# P-2000 is designed for the specific use of creating micropipettes and no other use is recommended.

This instrument creates items which should only be used in a laboratory environment for use on animal tissues. It is not intended to be used and should not be used in human experimentation or applied to humans in any way. This is not a medical device.

Do not open or attempt to repair the instrument without expressed and explicit instructions from Sutter Instrument Company. Extreme heat and high voltages are present and could cause injury.

Do not allow unauthorized and or untrained operatives to use this device.

Any misuse will be the sole responsibility of the user/owner and Sutter Instruments assumes no implied or inferred liability for direct or consequential damages from this instrument if it is operated or used in any way other than for which it is designed.